REVIEW

Current sunscreen controversies: a critical review

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Summary

Background/purpose: Sunscreens are believed to be a valuable tool in providing photoprotection against the detrimental effects of UV radiation, a known carcinogen. However, a number of controversies have developed regarding their safety and efficacy. This review summarizes the relevant studies surrounding these controversies.

Methods: Evidence of the prevention of skin cancer, an oft-cited reason for sunscreen use, was examined as it pertains to squamous cell carcinoma, basal cell carcinoma and melanoma. We also reviewed studies examining the effects of sunscreen on the synthesis of vitamin D, an essential nutrient whose role in health and disease continues to grow. Lastly, we analyzed studies surrounding the safety and toxicity of oxybenzone, retinyl palmitate and nanoparticles of zinc oxide (ZnO) and titanium dioxide (TiO_2).

Results: The overwhelming majority of available data is drawn from studies conducted using antiquated sunscreen formulations. Nonetheless, our research revealed that topical use of sunscreen protects against squamous cell carcinoma, does not cause vitamin D deficiency/insufficiency in practice and has not been demonstrated to adversely affect the health of humans.

Conclusion: Given the established benefits of UV protection, the use of sunscreens remains an important part of an overall photoprotective strategy. Future sunscreens with improved formulation should ideally offer superior protection. With increased usage of sunscreen by the public, continuous and vigilant monitoring of the overall safety of future products is also needed.

The detrimental effects of UV exposure from the sun have been well described in the literature. Both acute and chronic UV exposure can lead to sunburn, photocarcinogenesis, photoimmunosuppression and photoaging. Current methods of photoprotection include sun avoidance, seeking shade, use of protective clothing and the application of sunscreen. Of these, the use of sunscreens remain the most prevalent protection strategy used by the public.

However, concerns have emerged surrounding the safety and efficacy of sunscreens. Studies have been published assessing whether sunscreens prevent skin cancer, and conversely, confer an increased risk of melanoma, the most fatal form of skin cancer. Similarly, a growing body of literature demonstrating the beneficial effects of vitamin D on health outcomes has called into question the potential for vitamin D deficiency/insufficiency with sunscreen use.

Controversy has also developed regarding the possibility of adverse biological effects from various ingredients in sunscreens. Oxybenzone, an ingredient widely used in sunscreens, is purported to have a potentially disruptive effect on hormonal homeostasis. Retinyl palmitate, a compound used extensively in various cosmetic and personal care products, has received wide attention as a potential photocarcinogen. Lastly, safety concerns have also developed as a result of the inclusion of nanosized inorganic UV filters, zinc oxide (ZnO) and titanium dioxide (TiO_2), into sunscreens.

In this review, we attempt to clarify the above issues by critically analyzing the available evidence regarding these controversies.

Prevention of skin cancer

Squamous cell carcinoma (SCC) and basal cell carcinoma (BCC)

Routine use of sunscreen has been shown to be effective at reducing the development of both SCC (1, 2) and actinic keratoses (3–5), which are known precursors to SCC (6). However, a statistically significant protective benefit of

sunscreen has not been demonstrated against either BCC or malignant melanoma.

To date, a 1999 study published by Green *et al.* (1) is the only randomized controlled trial examining the effect of sunscreen use on the development of skin cancers. The study followed a group of 1621 Australians with a follow-up period of 4.5 years, from 1992 to 1996. Eligible subjects took a survey in 1992 and underwent a complete skin exam by a dermatologist in order to have skin cancers removed. Subjects were then randomized to either a no sunscreen group or a daily SPF 16 sunscreen group. The sunscreen group was instructed to apply sunscreen on the head, neck, arms and hands. Subjects were followed every 3 months and received free SPF 16 sunscreen. To ensure compliance, sunscreens were weighted on every visit. In 1994, and again in 1996, dermatologists who were blinded to the groups examined the subjects, and all clinically diagnosed skin cancers were histologically confirmed.

The investigators observed that sunscreen use had no effect on either the incidence of BCC or in the total number of BCC tumors. However, the overall incidence of SCC, in terms of people treated, was reduced by 12% among the participants randomized to the sunscreen group. This was not statistically significant because of the small number ($n = 22$) of individuals who developed SCCs. However, the investigators observed a statistically significant 39% reduction rate (95% CI 0.46–0.81) in the incidence of SCC tumors among the participants randomized to the sunscreen group.

To assess for potential latency of protective effects, a follow-up study to the above trial was published in 2006. In the follow-up study, all participants were followed for an additional 8 years (from 1996 to 2004) and agreed to report any subsequently diagnosed skin cancers to the researchers via local pathology laboratories. Over a period of 6 months, active participants completed monthly questionnaires about sunscreen use, outdoor sun exposure and the development of any new skin cancers.

Results from the 8-year follow-up indicated a rate reduction of 35% (95% CI 0.43–0.98) in the incidence of persons newly affected by SCC. The researchers also observed a rate reduction of 38% (95% CI 0.38–0.99) in the total number of SCC tumors. When the analysis of incidence and tumor number was limited to the late follow-up period (2001–2004), a rate reduction of 51% was observed for both SCC incidence (95% CI 0.28–0.83) and SCC tumor number (95% CI 0.27–0.87), respectively. No statistically significant reductions were observed in the incidence of BCC or total number of BCC tumors with daily sunscreen use. However, there was a nonsignificant 25% reduction [relative risk (RR) 0.75, 95% CI 0.49–1.14] in the incidence of BCC tumors in the late follow-up period among daily sunscreen users when compared with controls. It remains to be determined whether a sunscreen with higher SPF value would have further augmented this trend of decreasing BCC tumor incidence.

Melanoma

Evidence demonstrating the protective effect of sunscreen in the prevention of melanoma is inconclusive. To date, only case–control studies have been published, all of which are subject to recall bias of sunscreen use. At the time of this review, there have been no randomized controlled trials conducted examining the effects of sunscreens in preventing malignant melanoma. As a result, we provide reviews of two meta-analyses published on this topic.

A meta-analysis published in 2002 by Huncharek and Kupelnick (7), examined data from 11 case control studies published between 1966 and 1999. The researchers pooled data from studies in which each compared a particular frequency of sunscreen use with no history of sunscreen use. Overall, the studies compared people who had 'never' used sunscreen to those who had 'ever,' 'often,' 'regularly' or 'always' used sunscreen. After reviewing the data from all 11 studies, the overall RR was a statistically nonsignificant 1.11 (95% CI 0.37–3.32), indicating that the researchers failed to find a difference in the risk of developing melanoma when people who had 'ever,' 'regularly,' 'often' or 'always' used sunscreen were compared with people who had 'never' used sunscreen.

The second meta-analysis published in 2003 by Dennis *et al.* (8), pooled results from 18 case–control studies published between 1966 and 2003 which reported data on sunscreen use before melanoma diagnosis. This meta-analysis included all 11 of the case–control studies examined in the first meta-analysis cited above. Dennis and colleagues organized the data from the 18 case control studies into cases of people who had 'ever' used sunscreen and compared with cases of people who had 'never' used sunscreen. The number of studies which reported an increased risk of developing melanoma with sunscreen use was nine, while the number of studies reporting a decreased risk of developing melanoma with sunscreen use was seven. Finally, two of the studies showed no change in the risk of developing melanoma with sunscreen use. The combined odds ratio was 1.0 (95% CI 0.8–1.2), indicating that sunscreen use had neither a protective nor harmful effect on the development of melanoma when compared with no sunscreen use. When the data analysis was limited to studies which adjusted for sun sensitivity (i.e. those people who are more likely to use sunscreen and are at higher risk for melanoma), sunscreen reduced the odds ratio to 0.9 (95% CI 0.7–1.2), suggesting a mild protective effect. However, this result was not statistically significant.

A total of nine case–control studies examined by the two meta-analyses found an increased risk of developing melanoma with sunscreen use when compared with no use. There are several factors that may have contributed to this outcome in the case–control studies. First, from a study design perspective, few of the studies controlled for confounding factors that are important determinants in the development of melanoma, such as intermittent sun exposure (9), history of sunburns (10) and childhood UV exposure (11). Second, the behavior patterns of consumers when applying sunscreens may also explain the increased risk. Instead of applying sunscreen at a concentration of $2\,mg/cm^2$ (12), most individuals apply only 0.39–1.5 mg/cm^2 in practice (13–17) resulting in an effective SPF approximately 1/3 that of the labeled SPF (18). Third, at the time that these studies were conducted, individuals used

sunscreens with relatively low SPF (i.e. low UVB protection) and little to no UVA protection. UVA irradiation generates reactive oxygen species (19, 20), which can damage DNA (21) and are involved in all stages of cutaneous carcinogenesis (22).

Although no studies have shown conclusively that sunscreen diminishes the risk of melanoma development, indirect evidence in human studies exists. Numerous studies have demonstrated an increased risk of developing melanoma with increased numbers of acquired melanocytic nevi (23–31). As a surrogate marker for an increased risk of developing melanoma, acquired melanocytic nevi have been the focus of much research. To date, however, the only randomized controlled trial examining the role of sunscreen in the development of nevi in children was published in 2000 (32). The researchers found that consistent use of SPF 30 sunscreen was associated with a significant reduction in the rate of developing melanocytic nevi over a 3-year period (median counts, 24 vs. 28, $P = 0.048$). A second study was published by this group using the same data to examine the effects of sunscreen use on the development of nevi in children by anatomic site and nevi characteristics. The data again demonstrated that sunscreen use diminished the incidence of nevi on sun-exposed areas of the body, especially among freckled children (33). As one of the most rigorous study designs available, these results provide important evidence for the protective effect of sunscreens against the development of a risk factor for melanoma.

In conclusion, the evidence currently available indicates that regular sunscreen use provides prolonged protective benefit in preventing SCCs. However, while there is no demonstrated benefit of regular sunscreen use in reducing the incidence of BCC development, a trend toward reduced incidence of BCC tumors among sunscreen users has been observed. Furthermore, data regarding the preventive effect of sunscreen against melanoma remains inconclusive. One important limitation of the available evidence, however, is that most of the studies were conducted during the 1970s and 1980s when only sunscreens with low SPF and little to no UVA protection were available. Modern sunscreens have corrected these shortcomings. However, long-term studies using these newer sunscreens are not yet available. Moreover, it may take many years or decades to determine the potential protective effects of improved sunscreen formulations. Thus, it is reasonable to conclude that in conjunction with better education, future studies using these sunscreens may demonstrate a preventive effect.

Sunscreen-induced vitamin D deficiency

Overview

As one of the essential vitamins, vitamin D plays several roles in the development and maintenance of physiological homeostasis throughout life. Endogenous synthesis, beginning with cutaneous exposure to UVB radiation, is the primary means of accumulation in the human body (34–38). The pathway for this process has been well described elsewhere (39). Briefly, upon exposure to UVB radiation, 7-dehydrocholesterol is photoisomerized in the skin to precholecalciferol, or previtamin D3. This molecule is then converted to cholecalciferol, or vitamin D3, and stored within the body's fat cells. When physiologic demands are sufficiently high, vitamin D3 then enters the circulation to be further modified in two hydroxylation reactions. The first of which occurs in the liver, converting cholecalciferol to calcidiol, also known as 25-hydroxycholecalciferol (25(OH)D). The second hydroxylation occurs in the proximal tubules of the kidney, generating the active form of vitamin D also known as calcitriol or 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$). While $1,25(OH)_2D$ is the active form of vitamin D, it is 25(OH)D that is measured in the serum to determine vitamin D status.

Over the last several years, vitamin D has received increasing attention from the general public, medical professionals and research communities. While the importance of vitamin D in the development and maintenance of musculoskeletal physiology has been known for some time, a growing body of scientific literature has described many beneficial roles for vitamin D in health. As a result, guidelines regarding optimal daily vitamin D intake have undergone revision by numerous health organizations. The current recommended dosages range from 400 to 1000 IU/day (34–38).

While the recommended levels of vitamin D intake remain under debate, none of the current guidelines account for vitamin D synthesis via cutaneous exposure to sunlight. However, several studies suggest that UV exposure, specifically UVB, is the primary determinant of vitamin D status (40–44). As the benefits of vitamin D become more resonant, the UVB-blocking property of sunscreens has garnered increasing concern about the subsequent effects on vitamin D levels. Attention has centered on the potential for sunscreen use to result in significantly diminished production of vitamin D, resulting in deficiency/insufficiency of this important molecule.

Effects on vitamin D levels

Indeed, studies have reported the inhibition of cutaneous vitamin D synthesis with sunscreen use (45–47). A study published in 1987 by Matsuoka *et al.* (45) showed that a single application of a common ingredient in sunscreens, *para*-aminobenzoic acid (PABA), was capable of inhibiting synthesis of vitamin D, both *in vivo* and *in vitro*. Work published a year later by the same author reaffirmed the inhibitory effects of sunscreen on vitamin D synthesis. Researchers compared serum levels of 25(OH)D in patients with a history of skin cancer who had used a PABA-based sunscreen over the previous year to controls who did not use sunscreen regularly over the previous year (and, importantly, who did not have a history of skin cancer). They found that sunscreen users had a mean serum 25(OH)D level that was $< 50\%$ that of nonsunscreen-using controls: 40.2 ± 3.2 vs. 91.3 ± 6.2 nmol/l ($P < 0.001$), respectively (46). Of worthy note, the researchers did not have baseline measurements of 25(OH)D from either group. Furthermore, levels of UV exposure were not controlled for in either group. Behavioral differences might also have generated confounding effects given that the study group was comprised of patients who had a history of skin

cancer. Their behavior patterns toward UV exposure might be expected to vary greatly from the controls that had no history of skin cancer, tending toward increased UV protection and avoidance. A third study by Matsuoka *et al.* (47) on this subject published in 1990 found that whole-body application of SPF 15 sunscreen completely blocked the production of vitamin D after exposure to UVB radiation. These results were confirmed by Farrerons *et al.* (48) who found that sunscreen had statistically significant suppressive effects on 25(OH)D levels at three of the four time points measured by the researchers, when compared with no sunscreen use. However, the researchers concluded that while statistically significant changes were seen in 25(OH)D levels among sunscreen users when compared with controls, the decreases were not sufficient to induce significant alterations in the bone metabolic markers followed.

While the implications of the above data from controlled settings are suggestive of a suppressive effect of sunscreen on the cutaneous synthesis of vitamin D, similar results have not been borne out in studies conducted on a broader-scale reflecting the use of sunscreen in real life settings. Results from the first randomized controlled trial to assess this relationship were published by Marks *et al.* (49) in 1995. The study was conducted among 113 people over a 7-month period in Australia during which subjects were assigned daily application of either a SPF 17 sunscreen or a placebo cream. Despite consistent application of sunscreen in the study group, the results failed to show a significant difference in serum 25(OH)D levels between people who used SPF 17 sunscreen when compared with people who used the placebo cream: 11.8 nmol/l (95% CI 7.6–15.9) vs. 12.8 nmol/l (95% CI 8.4–17.1), respectively ($P < 0.001$). The researchers concluded that the participants who used the SPF 17 sunscreen received sufficient UV exposure to allow adequate vitamin D production to occur. This finding is consistent with data reported by Harris and Dawson-Hughes (50) in their questionnaire-based study of participants living in Boston, MA, in which the researchers found that sunscreen use was not correlated with the serum level of 25(OH)D.

In contrast, other studies have shown an increase in serum levels of 25(OH)D with sunscreen use. A study conducted among 49 older adults in Arizona by Kligman *et al.* (51) showed that serum levels of 25(OH)D were positively correlated with sunscreen use. Similarly, a questionnaire-based study conducted by Kimlin *et al.* (52) reported data from 124 adults living in Australia regarding the effects of sun protection and exposure behaviors on vitamin D status. The results demonstrated a trend of increasing 25(OH)D serum levels with increased sunscreen use, most prominently among users who applied sunscreen to the face. However, the association between sunscreen use and levels of 25(OH)D was not statistically significant. The positive association between 25(OH)D levels and sunscreen use observed in these two studies may be in some part a function of altered behavior patterns. Thieden *et al.* (53) reported that sunscreen use was directly related to the intention one had for excess UV exposure and that those who used sunscreen exposed themselves to solar radiation more frequently and for longer periods of time. Therefore, the increase in vitamin D levels observed in both of these studies could be due to an increased UV exposure associated with sunscreen use.

Conclusion

Considerable overlap exists between the UV absorption profiles of sunscreens and the action spectrum for vitamin D synthesis (54, 55). In theory, correct usage of sunscreens should significantly reduce vitamin D levels. However, this is not the case in practice. In fact, several studies have demonstrated that sunscreens are rarely applied correctly, in the right dosages and with appropriate frequency (13–17, 56). Therefore, under real-world conditions it is likely that the improper use of sunscreen and/or increased exposure time, result in production of vitamin D among sunscreen users. Lastly, it is important to note that the level of cutaneous vitamin D production from UVB exposure is also influenced by seasons, latitude, obesity level and age. Despite the potential inhibition of cutaneous synthesis with sunscreen use, sufficient levels of vitamin D can be achieved from dietary supplementation.

Safety of oxybenzone in sunscreen

Oxybenzone (benzophenone-3) belongs to the class of aromatic ketones known as benzophenones, which provide broad-spectrum UV coverage. The molecule was first approved for use by the Food and Drug Administration (FDA) in the early 1980s and has grown to become one of the most widely used organic UVA filters in US sunscreens today. As a photoprotective agent, it has an absorption profile spanning from 270 to 350 nm with absorption peaks at 288 and 350 nm. Despite established photoprotective effects, a number of important issues have arisen surrounding the use of oxybenzone in sunscreen.

Aside from its photoallergic potential (57–61), major concerns regarding its systemic absorption profile and its potential as an endocrine disruptor have engendered heated debate regarding the overall safety of this molecule. Systemic absorption of oxybenzone has been well demonstrated (62–65), and the prevalence of exposure in the general U.S. population is estimated to be as high as 96.8% (66). The molecule is predominately excreted via urinary and possibly fecal routes (62–65, 67–69), and studies with rats have demonstrated that oxybenzone and/or its metabolites accumulate primarily in the liver, kidney, spleen and testes and to a lesser extent in the intestine, stomach, muscle, heart and adrenal glands (69). In this section, we review the evidence specifically related to this issue.

In vitro studies

A number of *in vitro* studies have demonstrated estrogenic and anti-androgenic activity of oxybenzone. Nakagawa and Suzuki (70) reported an increase in the estrogenic activity of oxybenzone metabolites (relative to the parent compound, oxybenzone) in human breast cancer cells. Further work with human breast cancer cells was published by Ma *et al.* (71) and Heneweer *et al.* (72) demonstrating that oxybenzone exhibited

antiandrogenic activity and estrogenic effects, respectively. Schlumpf *et al.* (73) also published work in 2001 demonstrating an increase in the transcription of an estrogen-regulated gene in human breast cancer cells exposed to oxybenzone. The same author published data in 2004 demonstrating binding of estrogen receptors by oxybenzone in competition experiments with radiolabeled estradiol, as well as inhibition by oxybenzone of dihydrotestosterone-dependent gene activation in breast cancer cells (74).

In vivo studies

In 2001, Schlumpf *et al.* (73) assessed the estrogenic effect of oxybenzone via an uterotrophic assay in rats. In this protocol, the estrogen effect was measured by removing and weighing the uterus of rats. The researchers administered oxybenzone to immature rats after dissolving the compound in acetone or 99% ethanol. The resulting solution was then mixed into chow and the solvents were subsequently allowed to evaporate. The chow was then fed to 21-day-old rats for a period of 4 days. At the end of this period, the rats were euthanized and the uteri were removed and weighed. Of worthy mention, no acute toxicities were reported in the rats during the treatment period. The data demonstrated an increase of 23% in the uterine weight of rats exposed to oxybenzone. It is important to note that the oral dosage used to achieve the statistically significant increase in uterine weight was >1 500 mg/kg/day, an astronomically high dose.

Human studies

The above referenced *in vitro* and *in vivo* studies raise interesting questions regarding the potential effects of topically applied oxybenzone on endogenous reproductive hormones. However, in the hierarchy of toxicological studies, the gold standard for assessing risk to human health is a clinical study with human volunteers. One such study was performed by Janjua *et al.* (65) in 2004. In this single-blinded study involving 15 young males and 17 postmenopausal females, researchers assigned whole-body topical application of cream formulations containing oxybenzone at concentrations of 10% (wt/wt). The formulations were applied at a dose of 2 mg/cm^2. For the duration of the study, participants agreed to avoid exercise, sunbathing, caffeine, alcohol, or nicotine. As expected, systemic absorption of oxybenzone was observed in both males and females. In the women, oxybenzone reached a maximum plasma concentration of 200 ng/ml 3–4 h after whole-body application while among the male group oxybenzone peaked at 300 ng/ml after approximately the same amount of time. After 24 and 96 h, the plasma concentration for both groups did not differ, indicating that oxybenzone did not accumulate in plasma. Rather, the researchers found that it was excreted in the urine.

Among both females and males, the authors observed small but statistically significant differences in three of the six serum hormones measured (inhibin B and estradiol in males as well as testosterone in both males and females) between sunscreen containing oxybenzone and vehicle cream alone. Within the first four hours of applying a sunscreen containing oxybenzone, males demonstrated slightly lower levels of estradiol and testosterone, while inhibin B was slightly increased. Females showed a slight decrease in testosterone within the first 24 h. However, after 4 days of daily topical application no significant differences in the measured serum hormones existed between treatment or control groups among either males or females. The authors ultimately concluded that the differences in hormone levels were not related to the sunscreens containing oxybenzone.

In conclusion, systemic absorption of oxybenzone after topical application in both humans and animals has garnered significant attention. It is important to note that systemic absorption did not result in clinically significant perturbations of hormonal homeostasis in humans. Indeed, acute toxicity has not been reported in any of the *in vivo* or human studies published to date. While more work remains to be performed in this area, the available evidence does not demonstrate biologically significant hormonal disruption with topical application of oxybenzone in humans.

Safety of retinyl palmitate in sunscreens

Over the past 20 years, retinyl palmitate has found widespread use in cosmetic and sunscreen products. As of 2000, data from the FDAs Voluntary Cosmetics Registration Program indicate that the number of retinyl palmitate-containing formulations in the United States has increased to 667, up from 355 in 1992. In addition to cosmetic and sunscreen products, retinyl palmitate has also been approved by the FDA for use in a wide variety of over-the-counter and prescription drugs. It is also worthy to note that retinyl palmitate is commonly found as a food additive in the United States (e.g. to fortify low-fat milk, dairy products and breakfast cereals). Recently, media organizations have touted retinyl palmitate in sunscreen as having photocarcinogenic potential, calling into question the safety of its use in sunscreens. A critical analysis regarding the photocarcinogenic potential of retinyl palmitate has been reviewed by Wang *et al.* (75), and we will briefly summarize their findings herein.

Retinol (vitamin A) is an essential nutrient, which plays important roles in many biologic functions. In human skin, retinol is stored as retinyl palmitate. The compounds exhibit an interchangeable relationship with other retinoids, depending on the biochemical environment. When metabolic demands are sufficiently high, both retinyl palmitate and retinol are converted to active retinoic acids – all of which share similar pharmacologic and toxicologic profiles (76–78). Furthermore, when compared with retinyl palmitate in human skin, retinyl palmitate in sunscreens has the same pharmacological, biological and toxicological profiles.

A number of studies have been published by the FDA regarding retinyl palmitate. Of the eight *in vitro* studies published by the FDA from 2002–2009, four demonstrated the generation of reactive oxygen species by retinyl palmitate when exposed to UVA radiation (79–82). The generation of free radicals and their

subsequent mutagenic potential have garnered understandable concern. However, when considered in the context of the antioxidant milieu found in human skin, the relevance of these findings becomes questionable. The capacity to quench reactive oxygen species is magnified by the complex network of antioxidants found in the normal human biochemical environment. In conjunction with both enzymatic antioxidants (e.g. catalase, peroxidase, superoxide dismutase and glutathione reductase) and nonenzymatic antioxidants (e.g. vitamins C and E), vitamin A can neutralize harmful free radicals. In the isolative environment of laboratory study, however, cooperative interactions among other antioxidants are absent. As such, the protective properties of a single antioxidant are quickly depleted and may even become pro-oxidative when exposed to harmful stimuli. Furthermore, many antioxidants are inherently unstable if not properly formulated, preventing the full spectrum of enzymatic and nonenzymatic antioxidants from acting to reduce the pro-oxidative effects observed in these *in vitro* experiments.

To assess the carcinogenic potential of retinyl palmitate, the FDA's National Toxicology Program (NTP) conducted a large study using SKH-1 hairless mice. While the results from this study have not yet been published in a peer-reviewed forum, the preliminary data are available for review online (83). In this study, SKH-1 hairless mice received two different concentrations of retinyl palmitate (0.1% and 0.5%), with controls receiving a vehicle control pH 7 cream. The animals were then irradiated with UV doses of 6.75 and 13.7 mJ/cm^2 and subsequently assessed for photocarcinogenesis. In the groups irradiated with low-dose (6.75 mJ/cm^2) UV radiation, retinyl palmitate induced higher incidences of malignant lesions at concentrations of both 0.1% and 0.5%, when compared with the vehicle control pH 7 cream. However, only the group exposed to retinyl palmitate at a concentration of 0.5% showed a statistically significant increase. In the groups exposed to high-dose (13.5 mJ/cm^2) UV radiaton, no statistically significant difference in the incidence of malignant lesions was observed between the vehicle control group and the group exposed to either 0.1% or 0.5% retinyl palmitate. Therefore, the study failed to demonstrate conclusively that the combination of retinyl palmitate and UV is photocarcinogenic. Of worthy note, the thinner epidermis of the mice used in these studies allows for increased penetrance of UV radiation. Additionally, these mice are known to have a higher propensity to develop skin cancer. These intrinsic qualities suggest that data generated from these animal studies should be examined in context and caution should be exercised in extrapolating the relevance of these findings to humans.

While published data on the photocarcinogenic potential of retinyl palmitate in humans are lacking, evidence from 40 years of use in clinical medicine provides a powerful basis from which to question the notion that retinyl palmitate in sunscreen is photocarcinogenic. Clinically, retinoids are used by dermatologists in two major areas of therapy. First, oral retinoids have been used with great success to prevent skin cancers in populations who are at high-risk, such as patients with xeroderma pigmentosum (84) and immunosuppressed patients (e.g. organ transplant) (85). Second, dermatologists commonly prescribe topical retinoids in the management of skin disorders such as acne, psoriasis, photoaging, cutaneous T-cell lymphoma and a variety of other skin conditions. Among patients treated with topical or oral retinoids, no published data exist to date suggesting that these medications increase the risk of skin cancer.

In conclusion, the available evidence from *in vitro* and animal studies fails to demonstrate convincing evidence indicating that retinyl palmitate imparts an increased risk of skin cancer. Furthermore, while no human data examining this relationship are available, decades of clinical observations support the notion that retinyl palmitate is safe for use in topical applications such as sunscreens.

Safety of nanoparticles in sunscreen

Nanoparticles in sunscreen

The use of inorganic UV filters (i.e., titanium dioxide (TiO_2) and zinc oxide (ZnO)) in sunscreens has several advantages (e.g. photostability and low photoallergic reaction rate) over organic UV filters, such as avobenzone. While the photoprotective benefits of both TiO_2 and ZnO have been known for decades, earlier generations of inorganic filters were comprised of large particles, producing an opaque and white appearance on skin. In addition to cosmetic drawbacks, widespread adoption of early formulations containing TiO_2 or ZnO was further hindered by their poor dispersive properties and large particle size, which resulted in grainy and occlusive qualities.

Considerable effort has been made to overcome the cosmetic shortcomings of these inorganic UV filters by progressively minimizing the particle size into the nanorange (< 100 nm). Nanoparticles are known to exhibit different chemical, mechanical, electrical and optical properties than the standard-sized particles. From this, the nanoscaled versions are postulated to also exhibit altered biological properties, which may have negative health implications. The recent integration of TiO_2 and ZnO nanoparticles into sunscreens has raised interesting questions regarding the potential for dermal penetration, systemic absorption and subsequent toxicity. An analysis of the available evidence, however, fails to demonstrate toxicity of these products after cutaneous application to healthy, intact skin.

Epidermal penetration

Initial concerns were based on the prediction that the nanosized ZnO and TiO_2 would penetrate the skin barrier and exert toxic effects on tissue in the deep layers of the skin. A review of the currently available evidence does not demonstrate that nanoscaled UV filters penetrate living cells. Several *in vitro* and *in vivo* studies using both animal and human skin have shown that penetration of nanoscale ZnO or TiO_2 is limited to the stratum corneum (86–95), thereby precluding systemic absorption. Incidentally, this is similar to the distribution seen in conventionally sized ZnO or TiO_2. It is also important to mention that nanoparticles tend to aggregate once incorporated into

the ingredients of a sunscreen formulation. The final sizes of these aggregates exceed the 100 nm limit.

In vitro studies of toxicity

The conventionally sized particles of both TiO_2 and ZnO have been classified as biologically inert, as neither have significant toxicity profiles. Both ZnO and TiO_2 have been used in various products for decades. TiO_2 is one of the most widely used white pigments in use today. Its opacity is utilized to enhance the whiteness of products such as toothpaste, lotion, skim milk, cottage cheese, medicines, etc. In addition to being used for its pigment properties, ZnO has also been used extensively in a variety of other applications. ZnO is a component in many baby powders, anti-dandruff shampoos, barrier creams, etc. It is also found as an ingredient used to fortify several foods, including some breakfast cereals.

Despite the safety records, the potential for nanoparticle counterparts to cause systemic toxicity has generated legitimate concern. Among the mechanisms of toxicity, the development of free radicals is often cited. In fact, reports have demonstrated that nanoparticles of these compounds may have the potential to generate DNA-damaging reactive oxygen species when exposed to UV radiation (96). In 1997, Nakagawa *et al.* (97) conducted an *in vitro* study using murine and hamster cells to examine the potential photogenotoxicity of TiO_2. The researchers exposed mice leukemia, and Chinese hamster lung cells, to TiO_2 particles. They then challenged the cells with UV irradiation and subsequently assessed the chromosomes of these cells for any structural changes. The results showed that TiO_2 particles could act as a chemical mutagen after exposure to UV radiation. Other *in vitro* studies assessing the photogenotoxicity of both TiO_2 and ZnO have demonstrated similar results (98, 99).

In 2006, Dufour *et al.* (100) conducted an *in vitro* study assessing the purported induction of DNA damage by ZnO in the presence of UV radiation. The researchers exposed Chinese hamster ovary cells to micronized ZnO (mean particle size < 200 nm) while also varying the timing of UV radiation received by the cells. Cells exposed to ZnO were either: irradiated before being treated with ZnO, irradiated after being treated with ZnO or placed in the dark after being treated with ZnO (i.e. received no UV irradiation). The study found that the incidence of breakages or disruptions to the chromosomes did not vary as a function of cytotoxicity between cells irradiated in the absence of ZnO treatment when compared with cells irradiated while being treated with ZnO. This suggested that the DNA-damaging effects were not a result of UV-activated ZnO damage. Rather, they concluded that the chromosomal effects resulted from a UV-mediated increase in the susceptibility of the hamster cells to ZnO.

In 2000 (101) and 2003 (102), the Scientific Committee on Emerging and Newly Identified Health Risks delivered opinions on the use of TiO_2 and ZnO in cosmetics, respectively. They concluded that topical application of either compound did not result in toxicity or other adverse effects. An extensive study published in 2007 (103) by the producers of TiO_2-containing sunscreens assessed the effects of this compound *in vitro*. The study evaluated the phototoxic, genotoxic, photogenotoxic and cytotoxic potential of both nano- and microsized particles of TiO_2 on mammalian cells. The researchers reported data demonstrating that TiO_2, at both the micro- and nanoscale, had no adverse effects on mammalian cells. More recently, *in vitro* data published by Hackenberg *et al.* (104) in 2010 demonstrated that nanosized (15–30 nm) TiO_2 particles had no cytotoxic or mutagenic effects in human blood lymphocytes after 24 h of exposure at any of the four concentrations assessed by the researchers (highest 200 mcg/ml).

Conclusion

The increasing ubiquity of these nanocompounds in personal care and cosmetic products makes safety research especially relevant. Much concern have been voiced that the integration of nano-material technology into everyday formulations has outpaced the body of research evaluating their safety.

Currently, the FDA does not have regulations in place regarding the labeling of products containing nanoparticles of TiO_2 and ZnO. Considerable data assessing the potential toxicity of these materials in sunscreens has been published to date, and the studies referenced above were performed in controlled environments on healthy, undamaged skin. It has been established that the stratum corneum is an effective barrier preventing the entry of nano-ZnO and -TiO_2 into deeper layers of the skin. Nonetheless, it remains to be determined whether a greater degree of penetration occurs through skin that is damaged, diseased or otherwise compromised. At the present time, however, the available data do not provide conclusive evidence demonstrating that damaged skin leads to an increased penetration of nanoparticles (105).

Summary

Sunscreens remain an effective tool in providing protection against the known carcinogenic effects of UV radiation. However, there are a number of controversies surrounding the safety and efficacy of sunscreens as a form of photoprotection. We have attempted to address these controversies by examining the available published evidence and providing relevant analyses. The body of literature surrounding these controversies is vast and we have aimed to assess the most pertinent data.

Central to evaluating the evidence regarding the effect of sunscreen on skin cancer and vitamin D production, are the contrasts between theoretical vs. real-world application behaviors as well as early vs. modern sunscreen technologies. Improper application of sunscreen and increased sun exposure with sunscreen use are established behavior patterns, which confound data relevant to both of these controversies. Furthermore, inasmuch as studies evaluating skin cancer prevention were conducted using low SPF formulations, potential remains for substantially improved protective effects

from modern sunscreens. Similar issues affect the assessment of sunscreen use on vitamin D such that proper and consistent use of modern sunscreens would decrease endogenous production of vitamin D.

Analysis of the evidence regarding toxicity from sunscreen ingredients requires consideration of a few factors. First, careful consideration should be made for the type of study from which conclusions are drawn. Much of the concern for human safety was extrapolated from *in vitro* and/or animal data. Studies with humans, which provide the greatest evidence strength, did not demonstrate significant toxicity from any of the sunscreen ingredients in question. Second, the concern for systemic toxicity of topically applied compounds is largely drawn based on the systemic absorption profiles of the compounds. It is important to note that systemic absorption does not equal toxicity. In fact, numerous synthetic compounds used in cosmetic and personal care products are systemically absorbed and subsequently excreted without incident.

Sunscreens will continue to be a highly popular form of photoprotection in the foreseeable future. As modern formulations of sunscreens grow increasingly more sophisticated, the potential for human harm will continue to be assessed. However, the body of evidence establishing the protective benefit of sunscreens from a known carcinogen, UV radiation, is grounds for continued use. Nonetheless, important questions remain and much research is still needed. Future studies with humans will need to be conducted under real-world conditions with modern sunscreens, before we can determine definitively the safety and efficacy of sunscreen. However, none of the data published to date conclusively demonstrate adverse effects on the health of humans from the use of sunscreens.

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